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3. SUPPLEMENTARY NOTES

14. ABSTRACT
During the most recent period the Center of Excellence continued to collect and process human breast cancer tissues for patients receiving chemotherapy for metastatic disease. These tissues have been annotated clinically, examined by the study pathologist for QC/QA purposes, entered into our data analysis system, and distributed to the appropriate laboratory investigators for evaluation. An initial analysis of patients with metastatic breast cancer treated with the antimetabolite prodrug capecitabine was performed, and revealed striking correlations between thymidylate synthase (as measured by fluorescence in situ hybridization) and outcome.

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DOD Final Report: Center of Excellence for Therapeutic Individualization in Breast
    Cancer
PI: George W. Sledge, Jr.

Introduction

The Center of Excellence for Therapeutic Individualization for Breast Cancer was
created with the intent of bringing together modern genomic, pharmacogenomics, and
proteomic technologies to bear on the problem of drug therapy for metastatic breast
cancer. The work centered around the collection of clinically annotated tissue from
patients with metastatic disease with known clinical outcome data. Using these tissues,
cutting edge technologies will be used to determine which patients are most likely to
benefit from therapeutic intervention for metastatic disease.

Body

This report will highlight the overall progress made by the Center of Excellence.
As noted in previous reports, there was a significant change in our overall approach to the
Center of Excellence for Therapeutic Individualization for Breast Cancer. Our initial
approach involved obtaining frozen tissue samples for all women entering the trial.
Obtaining these tissues in a timely fashion proved more difficult than we initially
predicted, due to regulatory issues that delayed trial participation and due to accrual
problems at clinical sites. Simultaneously, changes in technology have made it possible
to perform high-quality analyses on formalin-fixed, paraffin-embedded tissues. We
therefore shifted the focus of clinical trial material from fresh frozen tissues to formalin-

fixed, paraffin-embedded tissues (FFPET). This shift in focus has had important consequences for trial performance, accrual, and technology.

From a trial standpoint, we added our COE05 trial. This trial, our "Retrospective-Prospective" trial, has obtained FFPET samples from women who have died of metastatic breast cancer and on whom clinical response and time to treatment failure data were available. During the past year we completed tissue acquisition from participating HOG institutions. Because patients may have been exposed to more than one chemotherapy regimen during the course of their disease, patients can provide informative data for multiple agents.

We received FFPE samples associated with 112 patients from a prominent medical oncology consortium in Poland (headed by Dr. Jacek Jassem) to provide additional specimens for COE05; overall we have obtained 241 case-eligible samples for COE05. These included 98 patients receiving AC, 192 patients receiving capecitabine, 121 patients receiving vinorelbine, and 63 patients receiving gemcitabine. RNA extraction is ongoing, with genomic and proteomic analysis also ongoing.

In addition to patients accrued through COE01, the Center has also had the good fortune to identify other available tissue sets obtained through previously performed clinical trials. Predominantly these represent tissues obtained by Dr. Jenny Chang and her colleagues at Baylor University, and include patients treated in the neoadjuvant setting with doxorubicin plus cyclophosphamide (AC) or docetaxel., Because the AC samples

obtained in this fashion satisfied our requirements for this combination, we closed this arm (Arm A) of the COE01 study. RNA extraction, frequently requiring associated tissue microdissection, was performed on these tissues and tissues distributed for analysis. Total accrual for this study was 212, with 107 patients receiving doxorubicin + cyclophosphamide, and 105 receiving docetaxel.

COE03 was our prospective trial examining the novel farnesyl transferase inhibitor lonafarnib. As in COE01, prospective data and tissue collection is performed in association with a clinical trial in patients with advanced disease. Accrual to this trial was completed during the current period, and tissues obtained for analysis. Unfortunately, the response rate to this intervention was sufficiently low in our metastatic population that tissue analysis is not justifiable. This trial did provide useful Phase II information regarding the role of lonafarnib in metastatic breast cancer, demonstrating its lack of therapeutic activity.

Our Patient advocacy core has become a significant research center during the past year, engaging in outcomes research with large patient populations using the conjoint analysis technique (a technique borrowed from the consumer marketing literature) to analyze trade-offs between toxicity and benefit and patient prefereces related to these outcomes.

Publications

Posters

R. Audet, C. Shen, R. Duchnowska, K. Adamowicz, J. Zok, W. Rogowski, M. Litwiniuk, S. Debska, M. Jaworska, M. Foszczynska-Kloda, M. Kulma-Kreft, K. Zabkowska, S. Edgerton, K. Vang Nielsen, A. Thor, J. Chang, K. Miller, J.

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Detailed Report

Introduction

In the metastatic context, the current selection of specific breast cancer treatment depends on hormone-receptor and HER2 status. For those patients who are non responsive to endocrine therapy or possess tumors negative for hormone receptors, standard chemotherapy remains the treatment option with single agent regimens usually consisting of either anthracyclines, taxanes, cyclophosphamide, fluorouracile, capecitabine, vinorelbine, or gemcitabine [Oostendorp et al., 2011;Beslija et al., 2009]. Unfortunately, there is no specific recommendation at present for second-line treatment or further chemotherapy as no particular regimen has been shown to offer greater efficacy [Cardoso et al., 2011]. In fact, from the 60% of patients with early-stage breast cancer that will receive adjuvant chemotherapy, only 2-15% will ultimately derive benefit from treatment, while all treated patients will be exposed to toxic side-effects [Early Breast Cancer Trialists' Collaborative Group (EBCTCG), 2005].

The primary objective of pharmacogenomics is to develop markers able to address specific aspects of response and/or toxicity and help in the individualization of breast cancer therapy. Biomarkers can be broadly categorized either as prognostic when solely associated with clinical outcome and predictive when associated with the effectiveness of a specific drug. A prognostic marker is a unique molecular feature or set of features assembled as a signature which can separate populations of patients based on disease outcome in the absence of treatment or despite a non-specific treatment. A predictive marker is, on the other hand, a unique molecular feature or a signature of features that can separate patient populations based on clinical outcome derived from a specific targeted therapy. When a predictive marker has

been properly validated, it can help to identify patients most likely to expect to benefit, or be less susceptible to suffer side effects from a particular therapy.

The quest for reliable predictive biomarkers for cytoyoxic agents has been, and will certainly remain, a long and challenging enterprise. As we gain a better understanding of the weaknesses of the available methodologies, it is becoming increasingly evident that each step in the analysis process is critical with regard to accuracy, reproducibility, and predictive value of new markers or signatures [Sauter et al., 2009]. In order to minimize inaccuracies, complementary techniques have been selected in parallel to assess the usefulness of theses biomarkers to predict response of an individual patient to a specific therapy.

Three different strategies were used to identify markers or signatures for each cytotoxic agent used in the Center of Excellence (COE) breast cancer retrospective cohorts. 1) Based on the concept that potential biomarkers have a better chance of being linked to a clinical response, a set of biomarkers were identified as either targets of a particular cytotoxic agent or determinants of its metabolism (Table 1). Gene copy number or protein expressions, of some of these selected markers, were evaluated using fluorescent in situ hybridization (FISH) and immunohistochemistry, respectively. 2) Using an mRNA expression profiling microarray-based assay (WG-DASL; Whole Genome cDNA-mediated Annealing, Selection extension and Ligation), genes that show a high differential expression between groups of patients with either a good or bad prognosis for a particular chemotherapeutic agent were characterized with univariate methods. 3) A dataset containing gene identifiers and corresponding WG-DASL gene expression values was uploaded to the Ingenuity application (http://www.broadinstitute.org/gsea/index.jsp) and mapped to its corresponding object in Ingenuity's Knowledge Base. Sets of genes grouped either by similar functional attributes, common transcription factors driven expression, or

chromosomal proximity that were most significant to the clinical outcome data were identified using Ingenuity's Knowledge Base.

Patients selection

Patients included in this study were all adult females over 18 years of age with pathologically confirmed breast cancer and locally advanced or metastatic disease treated with one or more of the following treatment regimens.

Cohort A: doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 day 1 of every 21-day cycle

Cohort B: capecitabine 1000 mg/m^2 BID days 1-14 of a 21-day cycle

Cohort C: vinorelbine 25 mg/m^2 days 1, 8, 15 of every 28-day cycle

Cohort D: gemcitabine 1000 mg/m^2 days 1, 8, 15 of every 28-day cycle

Archival FFPE blocks (formalin-fixed paraffin embedded) were obtained, sectioned, and the resulting slices were either mounted on glass slides for fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC) analysis or kept in RNase-free tubes for RNA extraction and further WG-DASL or PCR-based analysis.

The main clinical endpoints were time-to progression (TTP) and progression-free survival (PFS). TTP was defined as the time from treatment to disease progression. PFS was defined as the time from treatment to disease progression or death, whichever occurred earlier.

Analyses for the Center of Excellence (COE) studies

Overview of analyses per protocol and treatment cohorts

Selected markers and the corresponding methodologies to evaluate them are summarized in Table 1 for each of the treatments of the COE cohorts. Biomarker selection rationale along with a summary of results for each cytotoxic treatments is given in the following sections.

Statistical analysis

Normalization: There are many sources of noise in microarray data. The dye, scanner, arrays, and pin groups which are used to print the spot can all affect the expression level observed. This made normalization a critical step to eliminate bias. However, over-normalization may eliminate true biological signals as well. In consideration of both aspects, we used median normalization throughout our analyses where the median of signals from different arrays is normalized to be the same.

Comparison step: For binary outcome (response vs. non-response), the Significance Analysis of Microarray (SAM) and Prediction Analysis of Microarray is used for feature selection and the control of false discovery rate (FDR). The two approaches allow one to control FDR by estimating the null distribution of the test statistic (e.g., T-statistic) through permutation or parametric modeling. The estimate is relative robust to deviation from standard normal, which is partially due to the correlation among those signals. We also used Cox proportional regression model for survival-type of outcome (e.g. TTP and PFS) and control the FDR.

Gene Ontology Analysis: To evaluate gene functions and categories defined by various criteria, Gene Ontology (GO) offers a convenient platform (http://www.geneontology.org/). For each of the three types of Gene Ontology

terms (molecular function, biological process and cellular component), hypergeometric distribution can be used to test the over/under representation of each term for the genes displaying differential expression between groups. A significant p-value then suggests that the very feature that defines the groups (e.g., response and non-response) might be correlated with the term under consideration (e.g., a specific biological function).

An alternative approach is the Gene Set Enrichment Analysis (GSEA software http://www.broadinstitute.org/gsea/index.jsp). In GESA, a set of pre-specified genes was constructed based on certain biological rationale (e.g. involved in the same biological process or pathways). The analysis seeks to assess whether or not the gene expression level for the set as a whole (e.g. summary measure of expression level of genes in the set) differs between the comparison groups. It has the advantage of detecting a set of small signals that would otherwise be difficult to detect.

Marker selection rationale for Cohort A: doxorubicin and cyclophosphamide

Molecular pharmacology and mechanisms of action

Cyclophosphamide is a prodrug that undergoes activation through phase I metabolism via the enzymes *CYP2B6*, *CYP3A4*, *CYP3A5* and *CYP2C9* into 4-hydroxy-cyclophosphamide, the active metabolite responsible for cyclophosphamide's alkylating properties [de Jonge et al., 2005;Huitema et al., 2000]. This molecule can be inactivated through a phase II conjugation with a thiol or sulfate via glutathione S-transferases (*GSTs*) or oxidized by the enzyme aldehyde dehydrogenase 1 (*ALDH1*) into carboxyphosphamide [Bunting et al., 1994].

Correlation between single nucleotide polymorphism in the activating enzyme *CYP3A4* or the metabolizing enzyme *GST1* and clinical outcome were suggested by small studies in breast cancer [DeMichele et al., 2005;DeMichele et al., 2007;Su et al., 2010] but it was only recently that an association between small nucleotide polymorphism and cyclophosphamide efficacy was observed in a larger cohort [Gor et al., 2010]. Although conceptually interesting, a direct link between the expression of these enzymes and clinical efficacy of cyclophosphamide-based chemotherapeutic regimens remains to be established.

The cancer stem cell hypothesis has fueled much research on *ALDH1* as a marker of breast cancer stem cells [Moreb, 2008] but it was also shown to be a predictor of response to cyclophosphamide in breast cancer patients [Sladek et al., 2002]. Although the number of patients was small, they were able to show that therapeutic outcome of cyclophosphamide-based chemotherapy corresponded to cellular *ALDH1A1* levels in 77% of cases [Sladek et al., 2002]. Indirect evidence is also available from many *in vitro* studies showing that *ALDH1A1* is directly linked to sensitivity to cyclophosphamide, consistent with its role in cyclophosphamide metabolism [Bunting and Townsend, 1996;Ekhart et al., 2008;Levi et al., 2008;Moreb, 2008;Sreerama and Sladek, 1997].

The anthracycline doxorubicin has been one of the most widely used agents in the treatment of breast cancer for the past quarter century. Anthracyclines have three principal mechanisms of actions: 1) they intercalate themselves between base pairs of DNA/RNA strands thereby inhibiting DNA and RNA synthesis, 2) they enhance the catalytic oxidation-reduction reactions, and 3) they inhibit topoisomerase II alpha (*TOP2A*).

Although evidence suggests that anthracycline-based regimens are significantly more efficacious than non anthracycline-based regimens in *HER2* positive patients but not *HER2* negative patients, some studies demonstrated that response to treatment in this group is not uniform [Pritchard et al., 2008]. This has led to the postulation that anthracyclines target *TOP2A*, with its gene neighboring the *HER2* gene on chromosome 17q12-21, may be the modulator of response to therapy. This interest was sparked by the finding that the *TOP2A* gene is frequently co-amplified with *HER2* [Jarvinen et al., 1999;Jarvinen et al., 2000;Zaczek et al., 2010]. However, this notion is complicated by the fact that *TOP2A* amplification does not strongly correlate with *TOP2A* protein expression [Corzo et al., 2007;Moretti et al., 2009;Oakman et al., 2009]. Besides *TOP2A* amplification, some authors have observed *TOP2A* deletions and found incidence levels ranging from 16 to 43% in *HER2* amplified tumors [Hicks et al., 2005;Jarvinen et al., 1999;Orlando et al., 2008].

Although recent data shows that *TOP2A* is also amplified in 27% of *HER2* negative tumors [Zaczek et al., 2012], previous data showing that *TOP2A* aberrations are found almost exclusively in HER2 amplified tumors and that both *TOP2A* deletions and amplifications can be found in the same tumor led to the theory of a cascade-type effect at 17q12-21 [Glynn et al., 2010]. These authors propose that *HER2* amplification is the first step in a series leading to an increased rate of *TOP2A* aberrations and possibly other surrounding genes. Coherent with the observed higher level of amplification for *HER2* compared to *TOP2A*, Nielsen *et al* recently showed that co-amplification of *HER2* and *TOP2A* is not the main mechanism behind aberrations seen in these genes and that different mechanisms may be involved [Nielsen et al., 2010].

Attempts to come up with a gene signature predicting benefits from anthracycline-based therapy are currently pursued and recent publications suggest that such a panel of biomarkers will necessarily involve many aberrant genes leading to altered protein expression and cellular regulation [Desmedt et al., 2011]. Recently, strategies to overcome the inherent noise in microarray data

were developed with the selection of genes with common features or their relation in the intracellular network [Garcia-Bilbao et al., 2012].

The present research aim is twofold; 1) shed some light on possible links between clinical efficacy of AC and *HER2* and *TOP2A* gene aberrations as well as their gene expression levels, 2) determine if a specific gene expression signature could be used as predictive marker for treatment outcome.

Results summary for Cohort A: doxorubicin and cyclophosphamide

Sixty adult female patients with pathologically confirmed locally advanced or metastatic breast cancer were treated with doxorubicin 60mg/m^2 and cyclophosphamide 600 mg/m^2 day 1 of every 21-day cycle. Archival FFPE specimens, taken before chemotherapy, were used to evaluate *TOP2A* gene status by FISH, *HER2* gene expression by IHC, and WG-DASL gene expression as described in the previous section.

Full statistical analysis is currently underway and will be subsequently published, but interim results suggest that *TOP2A* FISH gene copy number could be useful to identify patient populations most likely to benefit from an anthracycline-based therapy. These results should also shed some light on the link between *TOP2A* gene aberrations and altered gene expression.

Interim univariate expression analysis of key genes is also underway and will be published shortly but does not seem as promising as gene-set enrichment analysis (GSEA) in helping to predict clinical outcome. GSEA clustering by functional attributes, chromosomal location or common transcription factor driven gene expression provides important insights into complex gene expression changes related to the efficacy of chemotherapeutic agents. This novel approach holds the promise of facilitating the identification of gene sets enriched in tumors of patients with either more favorable or poorer outcomes when treated with anthracyclin regimens.

Markers selection rationale for Cohort B: capecitabine

Molecular pharmacology and mechanisms of action

The fluoropyrimidine nucleoside analogue 5-fluorouracil (5-FU) was originally developed as a cytotoxic agent over 50 years ago and is the standard treatment for a wide range of common solid tumors, including breast cancer. Attempts to increase the efficacy and tolerability of fluoropyrimidine treatment have led to the development of capecitabine (Xeloda™), a prodrug transformed into 5-FU preferentially in tumors (Figure 1). Capecitabine is now often used either alone or in combination with other drugs but, unfortunately reliable methods for selection of patients who have the best chance to benefit from capecitabine-based treatments are still lacking.

Capecitabine is activated at the tumor site by the enzyme thymidine phosphorylase (*TYMP*) [Miwa et al., 1998], which takes advantage of the fact that this enzyme is more highly expressed in tumor tissue [Takebayashi et al., 1996], including breast cancer [Kobayashi et al., 2005]. Capecitabine and its intermediate metabolite, 5'-deoxy-5-fluorouridine (5'-DFUR) are not cytotoxic but become effective only after conversion to 5-fluorouracil (5-FU) by *TYMP* as well as further transformations into fluorodeoxyuridine monophosphate (FdUMP) and fluorouridine triphosphate (FUTP) [Miwa et al., 1998]. Inhibition of the enzyme thymidylate synthase (*TYMS*) by FdUMP is considered to be the main mechanism of action of fluoropyrimidine, including capecitabine [Walko and Lindley, 2005].

TYMS is an important enzyme in pyrimidine metabolism which is crucial for *de novo* thymidine nucleotide synthesis used for DNA replication and cellular division [Peters et al., 1995]. Inhibition of *TYMS* occurs as a result of the formation of an inactive ternary covalent complex between *TYMS*, FdUMP, and 5-10 methylenetetrahydrofolate (CH_2FH_4). The stability of this ternary complex is

highly dependent on the availability of CH_2FH_4 or one of its polylglutamates [Houghton et al., 1982;Houghton and Houghton, 1983]. Dihydrofolate reductase (*DHFR*) is a key enzyme involved in folate metabolism and plays a role in the *de novo* pathway of pyrimidine biosynthesis that has been linked to the modulation of fluoropyrimidine treatments [Capiaux et al., 2003;Will and Dolnick, 1989].

Dehydropyrimidine dehydrogenase (*DPYD*) is the enzyme responsible for the first and rate limiting step in the catabolic conversion of 5-FU to inactive metabolites and decreases 5-FU levels within cells [Johnson et al., 1997;Lee et al., 2004;Lu et al., 1993]. Several studies have underlined the role of *DPYD* deficiency in the development of severe 5-FU toxicity and conversely *DPYD* overexpression is associated with resistance to these therapies [Kornmann et al., 2003]. Both elevated *DPYD* gene copy number and mRNA expression were linked to increased resistance to capecitabine and other 5-FU-based treatments in several human cells lines including breast [Kobunai et al., 2007].

Since *DPYD* is rate limiting for the catabolic pathway and TYMP is key to the production of active capecitabine metabolites, the *TYMP/DPYD* ratio has been frequently used to correlate with capecitabine or 5-FU efficacy. It was first shown that a high *TYMP* to *DPYD* ratio correlated with a high capecitabine efficacy and conversely a low *TYMP/DPYD* ratio was linked to resistance in a large number of xenograft models including breast [Ishikawa et al., 1998]. Recent immunohistochemical (IHC) data has shown that a higher *TYMP/DPYD* ratio correlates with better clinical response in a small cohort of breast cancer patients treated with capecitabine monotherapy [Honda et al., 2008].

Similarly, RT-PCR analysis of tumors from 22 breast cancer patients revealed that the patients expressing high levels of *TYMS* and *DPYD* were resistant to 5-FU, as opposed to the patients expressing low levels of *TYMS* and *DPYD* who were sensitive to this compound [Kakimoto et al., 2005]. Using IHC, it was shown that high levels of *TYMP* expression in tumors was a significant prognostic indicator of 5'-DFUR efficacy in breast cancer patients [Tominaga et al., 2002].

Therefore, the fluoropyrimidine pathway enzymes, *TYMP, TYMS, DPYD* and *DHFR*, were selected as potential candidate biomarkers that could be used to predict tumor response to capecitabine. Efforts have been made to select assays that would be easily accessible to clinicians in order to correlate gene copy number and gene expression profiles with disease state, therapy, and drug response.

Results summary for Cohort B: capecitabine

Newly developed FISH probes (Dako, Glostrup, Denmark) were used on 5µm FFPE tissue slices to investigate *TYMS*, *DHFR* and *TYMP* gene copy number. Hybridization signals were evaluated using either the ratio of red signals for *TYMS*, *DHFR* or *TYMP* to green signals for a reference sequence on the same chromosome or only the green signal from genes in at least 60 morphologically intact and non-overlapping nuclei. As these new probes have not being fully characterized, we did not apply a threshold as for the *TOP2A* FISH probes but categorized the results into high versus low gene copy number by the median.

TYMS is the primary target of capecitabine. Interim results presented at San Antonio Breast Cancer Symposium in 2011 show that higher *TYMS* gene copy number was significantly associated with a higher hazard ratio of both PFS and TTP in the overall patient population as well as in the *ER+* and *HER2-* subpopulations (Table 2 and Figure 2). *TYMS* gene copy number was also shown to be significantly correlated with its gene expression by DASL suggesting that multiple gene copies induce an increase in gene expression (Table 3). Although higher *TYMS* gene expression measured with DASL was also associated with increased hazard ratio both in the overall population, and in the *ER+* and *HER2-* subpopulations, they failed to reach the statistical significance level (Table 4 and Figure 3). These results suggest that an increased number of *TYMS* gene copies of this gene leads to an increase in its expression and is associated with a decreased benefit from capecitabine

therapy. The fact that *TYMS* DASL expression is not associated with outcome may reflect the limitations of RNA extraction from tissue containing many different cell types in addition to cancerous cells, thereby diluting the signal, a situation not encountered in FISH scoring performed exclusively in cancer cells.

Conversely, both PFS and TTP were not significantly different in the patients groups with a high or low *TYMP* gene copy number (Table 5 and Figure 4) neither was the DASL gene expression correlated with gene copy number (Table 3). Interestingly, higher *TYMP* gene expression measured with DASL was associated with significantly decreased hazard ratio in the overall population as well as in the *ER+* and *HER2-* subpopulations (Table 6 and Figure 5), consistent with *TYMP*'s role in activating capecitabine [Miwa et al., 1998] and the fact that it is amplified in tumors by post-transcriptional mechanisms [Toi et al., 2005].

Both PFS and TTP did not correlate with *DHFR* gene copy number whether in the overall patient population or in the different ER and HER2 subgroups (data not shown). Interestingly, a significant inverse correlation was observed between *DHFR* gene copy number and DASL expression (Table 3). A higher *DHFR* DASL expression was also associated with a significantly lowered hazard ratio for PFS but not TTP in the *ER+* subgroup (data not shown).

Microarray based WG-DASL gene expression data was also analyzed using a gene-set enrichment analysis (GSEA) looking at gene-sets grouped either by functional attributes, common transcription factors or chromosomal proximity. The GSEA software (http://www.broadinstitute.org/gsea/index.jsp) was used for the analysis and results will be available for publication in a near future.

Conclusions

TYMS:

1) Increased *TYMS* gene copy number measured by FISH was associated with reduced capecitabine benefit in the overall population and particularly in the *ER+* and *HER2--* subpopulation. 2) Protein expression of *TYMS* was significantly

correlated with gene copy number (GCN). 3) Although not significant, a similar trend was observed using DASL suggesting that FISH measured directly in tumor cells is more sensitive than an RNA pool including various cell types.

TYMP:

1) *TYMP* GCN was not associated with outcome. 2) *TYMP* GCN was not significantly correlated with expression confirming reports that RNA is amplified in tumors by post-transcriptional mechanisms. 3) High *TYMP* DASL expression was significantly associated with increased capecitabine benefit in the overall population (and particularly in the *ER+* and *HER2-* population) consistent with its role in activating capecitabine.

DHFR:

 1) *DHFR* GCN was not associated with outcome. 2) *DHFR* GCN was inversely correlated with expression. 3) High *DHFR* DASL expression was significantly associated with increased capecitabine benefit in *ER+* patients in line with its role in providing tetrahydrofolate necessary to inactivate the enzyme complex.

A similar correlation between a higher *TYMS* gene copy number and elevated protein expression was associated with decreased benefit from 5-FU adjuvant therapy in colorectal cancer patients [Jensen et al., 2008].

Markers selection rationale for Cohort C: vinorelbine

Molecular pharmacology and mechanisms of action

Vinorelbine (Navelbine®) is a semi-synthetic vinca-alkaloid that exhibits antimitotic activity by interfering with the dynamic equilibrium of tubulin [Lobert et al., 1996]. It inhibits tubulin polymerization and preferentially binds to mitotic microtubules causing cell death following a block in mitosis at G2-M [Binet et al.,

1989;Fellous et al., 1989;Goa and Faulds, 1994]. Like other vinca-alkaloids it may interfere with amino acid, cyclic AMP and glutathione metabolism as well as with calmodulin-dependent Ca-transport or cellular respiration [Galano et al., 2011]. Vinorelbine is metabolized via deacetylation, hydroxylation, dealkylation and oxidation leading to the generation of many secondary metabolites [de Graeve et al., 2008]. Although all of these interactions with intracellular elements represent possible determinants of vinorelbine efficacy, there are relatively few clinical studies addressing this question.

The most obvious and well-studied target of vinorelbine is beta tubulin III (Gene symbol: *TUBB3*). A recent study has shown that histocultures from lung tumors with high *TUBB3* protein levels exhibited greater chemosensitivity to vinorelbine than tumors with lower *TUBB3* levels [Hirai et al., 2011]. Conversely, other studies have shown that it is the up-regulation of *TUBB3* which is implicated in drug resistance and not pretreatment levels of *TUBB3* [Saussede-Aim et al., 2009].

Vinorelbine was shown to bind with such a high affinity to chromatin that it decreases its melting point and the principal drug binding site is thought to be the globular domain of histones [Rabbani-Chadegani et al., 2009;Rabbani-Chadegani et al., 2011]. Modifications of histones by methylation or acetylation has been shown to be a key element of gene transcription changes observed in many cancers, including breast [Stratmann and Haendler, 2012], and binding of vinorelbine to the histone complex could logically be involved in gene expression involved in the efficacy of this drug.

Our goal was to identify individual genes or gene sets whose expression may affect the efficacy of vinorelbine in breast cancer patients to better individualize therapy.

Results summary for Cohort C: vinorelbine

WG-DASL analysis

Forthy-three adult female patients with pathologically confirmed breast cancer and locally advanced or metastatic disease were treated with vinorelbine 25 mg/m^2 days 1, 8, 15 of a 28-day cycle. Gene expression was assessed in archival FFPE tissue using the microarray-based WG-DASL assay and correlated with TTP. Using GSEA, gene sets that share a common molecular function, chromosomal location, or regulation were identified in patients classified as having either a short (S) (n=25) or a long (L) (n=18) time to progression (TTP) divided by the median (72 days). GSEA software (http://www.broadinstitute.org/gsea/index.jsp) was used for the analysis.

Interim GSEA results presented at ASCO 2012 have shown that when genes were grouped according to similar molecular function; 16 out of a set of 43 genes involved in histone binding were enriched in group S (p = 0.002), consistent with higher expression in group S of *HIST3H2BB* and *HIST1H3I.* GSEA analysis of genes grouped according to common transcription factors has shown that 14 out of 47genes were enriched in group S (p = 0.004) including promoter regions that match c-fos serum response element-binding transcription factor and other promoter regions linked to histone expression as well as the cellular membrane pumps *P-gp/MDR1* involved in vinorelbine transport [Wong et al., 2006].

GSEA analysis of genes grouped according to chromosomal location has shown that in group S, genes were enriched on chromosome 11q21 (20 out of 45 genes p = 0.004) and on chromosome 12p12 (14 out of 22 genes p = 0.002). These chromosomal regions could represent "hot spots" were genes are over expressed following damage or rearrangement of DNA, but further studies are definitively needed in order to unravel the underlying mechanisms.

Conclusions:

GSEA suggest that there is an up-regulation of histone binding genes in patients deriving the least benefit from vinorelbine therapy, which is consonant with recent discovery of high affinity vinorelbine binding to histones [Rabbani-Chadegani et al., 2009;Rabbani-Chadegani et al., 2011]. The role of *P-gp/MDR1*

in extracellular transport and resistance to vinorelbine is well known and our finding of an increased transcription factor linked to their expression deserves additional scrutiny as our novel observations on chromosome 11q21 and12p12. DASL expression combined with GSEA highlights gene sets that correlate with clinical outcome and may lead to predictive markers of vinorelbine efficacy. Further confirmatory analysis is needed due to the limitation of small sample size and multiple comparisons

Markers selection rationale for Cohort D: gemcitabine

Molecular pharmacology and mechanisms of action

Gemcitabine (Gemzar®) is a cell cycle-dependent (S-phase-specific) deoxycytidine analogue frequently used in patients with solid tumors and must be first transported into the cell and phosphorylated to its active triphosphate form. Because gemcitabine is hydrophilic and does not readily cross plasma by passive diffusion in order to gain access to its intracellular targets it requires the presence of specialized membrane nucleotide transporters [Mackey et al., 1998].

Gemcitabine is taken up into cells via the family of human nucleoside transporter (hNT) including equilibrative (hENT) and concentrative (hCNT) members (Figure 6) [Veltkamp et al., 2008]. hENTs are capable of transporting pyrimidine and purine nucleotides both from outside and inside cells and are widely distributed in human cells. On the other hand, hCNTs can transport pyrimidines and purines across the cellular membrane against a concentration gradient and have generally a higher affinity for transport of nucleotides than hENTs (i.e., hCNT1 has a tenfold higher affinity for gemcitabine than hENT1) [Mackey et al., 1998]. hENT1 is known as member 1 of the solute carrier family 29 (gene abbreviation: SLC29A1). hCNT2 is officially known as member 2 of the solute carrier family 28 (gene abbreviation: SLC28A2).

Phosphorylation of gemcitabine by deoxycytidine kinase (dCK) is the first and rate-limiting step of the formation its active form, fluorodeoxycytidine monophosphate (dFdCMP) before being transformed in its main active metabolite 2'-2'-difluorodeoxycytidine triphosphate (dFdCTP), which is incorporated into DNA and inhibits DNA synthesis [Veltkamp et al., 2008].

Results summary for gemcitabine

Data is being compiled and will include immunohistochemistry analysis of *ENT*, *DCK* and *CNT* as well as expression analysis using either DASL or qRT-PCR for *ENT*, *DCK*, *CNT* and *RRM1* (table 1). These data will allow assessing changes both at the mRNA and protein levels and correlating them with benefits from gemcitabine chemotherapy. GSEA will also be performed and should hopefully bring new insights regarding the tumor characteristics that have an impact on clinical outcome of gemcitabine chemotherapy.

OVERALL SUMMARY

Marker discovery and development is a complex, time-consuming and expensive enterprise, which can be simplified greatly by the use of good quality archival specimens. Nevertheless, our interim data is promising and clearly shows that although pinpointing a particular phenotype associated with a clinical outcome is challenging, new integrated methods are available and holds the promise of better targeting classical chemotherapy.

Table 1: Overview of the selected analyses for each treatment cohort.

Protocol	FISH[1]			Immunohistochemistry[2]			WG-DASL[3]	Potential markers for qRT-PCR
COE-01 and COE-05								
cohort A: AC	TOP2			HER2			√	ALDH1A1, HER1, HER2, HER3, TOP2A,
cohort B: Capecitabine	DHFR	TYMP	TYMS				√	DHFR, DPYD, TYMP, TYMS
cohort C: Vinorelbine							√	STMN1, TUBB3,
cohort D: Gemcitabine				ENT	DCK	CNT	√	CNT, DCK, ENT, RRM1

Abbreviations: **AC**: **A**driamycin® (doxorubicin) and **C**yclophosphamide, **ALDH1A1**: Aldehyde dehydrogenase 1, **CNT**: Concentrative nucleoside transporter, **COE**: Center of excellence, **WG-DASL**: Whole Genome c**D**NA-mediated-**A**nnealing, **S**election, extension and **L**igation, **DCK**: Deoxycytidine kinase **DHFR**: Dihydrofolate reductase, **DPYD**: Dihydropyrimidine dehydrogenase, **ENT**: Equilibrative nucleoside transporter, **FISH**: Fluorescent In situ hybridization, **HER**: Human epidermal growth factor receptor, **qRT-PCR**: quantitative real time polymerase chain reaction, **RRM**: Ribonucleotide reductase M **STMN1**: Stathmin 1, **TOP2A**: DNA topoisomerase-2-alpha, **TUBB3**: Tubulin beta 3, **TYMS**: Thymidylate synthase, **TYMP**: Thymidine phosphorylase.

[1] FISH probes developed by Dako (Glostrup, Denmark) were used on 5µm FFPE tissue slices to investigate TOP2A, DHFR, TYMP or TYMS gene copy number. Hybridization signals were evaluated in at least 60 morphologically intact and non-overlapping nuclei. The TOP2A genes to reference sequence ratio was considered deleted <0.8, normal ≥0.8 and <2.0 and amplified ≥2.0. The DHFR, TYMP and TYMS gene copy number were dichotomized by the median.

[2] Antibodies for hENT, hCNT and dCK were synthesized in Dr. John Mackey's lab and used according to established protocols [Mackey et al., 2002;Hatzis et al., 1998]

[3] Total RNA was extracted from 5 µm thick FFPE sections using the EPICENTRE QuickExtract™ kit and converted into cDNA before being hybridized to Whole-Genome-cDNA-mediated Annealing, Selection extension and Ligation BeadChips (Illumina, Inc., San Diego, CA, USA) according to the manufacturer's instructions. The signals were processed with the Bead-Studio Gene Expression Module (Illumina, Inc., San Diego, CA, USA).

Table 2: Hazard ratio (HR) between thymidylate synthase (*TYMS*) gene copy number measured by FISH and progression free survival (PFS) and time to progression (TTP) in patients treated with capecitabine.

TYMS	PFS	TTP
	H R (p-value)	H R (p-value)
Overall population (n=75)	**1.86 (0.01)**	**1.76 (0.03)**
ER+ (n=40)	**2.73 (0.01)**	**2.46 (0.02)**
ER- (n=35)	0.81 (0.55)	0.81 (0.55)
HER2+ (insufficient number)	-	-
HER2- (n=59)	**2.07 (0.01)**	**1.94 (0.03)**

Table 3: Correlation between gene copy number measured by FISH and gene expression measured by DASL in patients treated with capecitabine.

Gene (patients)	Pearson (p-value)	Spearman (p-value)
TYMS (n=57)	**0.26 (0.049)**	0.25 (0.056)
TYMP (n=48)	0.24 (0.1)	0.11 (0.461)
DHFR (n=17)	**-0.64 (0.006)**	-0.41 (0.098)

Table 4: The impact of thymidylate synthase (*TYMS*) gene expression measured by DASL on progression free survival (PFS) and time to progression (TTP) in patients treated with capecitabine.

| *TYMS*	PFS H R (p-value)	TTP H R (p-value)
Overall population (n=73)	1.19 (0.16)	1.23 (0.15)
ER+ (n=40)	1.24 (0.22)	1.46 (0.07)
ER- (n=33)	1.09 (0.67)	1.05 (0.84)
HER2+ (insufficient number)	-	-
HER2- (n=60)	1.21 (0.15)	1.17 (0.34)

Table 5: The impact of thymidine phosphorylase (*TYMP*) gene copy number measured by FISH on progression free survival (PFS) and time to progression (TTP) in patients treated with capecitabine.

TYMP	PFS H R (p-value)	TTP H R (p-value)
Overall population (n=75)	1.12 (0.65)	1.08 (0.79)
ER+ (n=40)	0.92 (0.83)	0.84 (0.67)
ER- (n=35)	0.82 (0.62)	0.82 (0.65)
HER2+ (insufficient number)	-	-
HER2- (n=59)	1.13 (0.68)	1.08 (0.81)

Table 6: The impact of thymidine phosphorylase (*TYMP*) gene expression measured by DASL on progression free survival (PFS) and time to progression (TTP) in patients treated with capecitabine.

TYMP	PFS H R (p-value)	TTP H R (p-value)
Overall population (n=75)	**0.17 (0.007)**	0.26 (0.06)
ER+ (n=41)	**0.1 (0.04)**	0.35 (0.40)
ER- (n=33)	0.22 (0.07)	0.25 (0.09)
HER2+ (insufficient number)	-	-
HER2- (n=60)	**0.17 (0.03)**	0.25 (0.12)

Figures
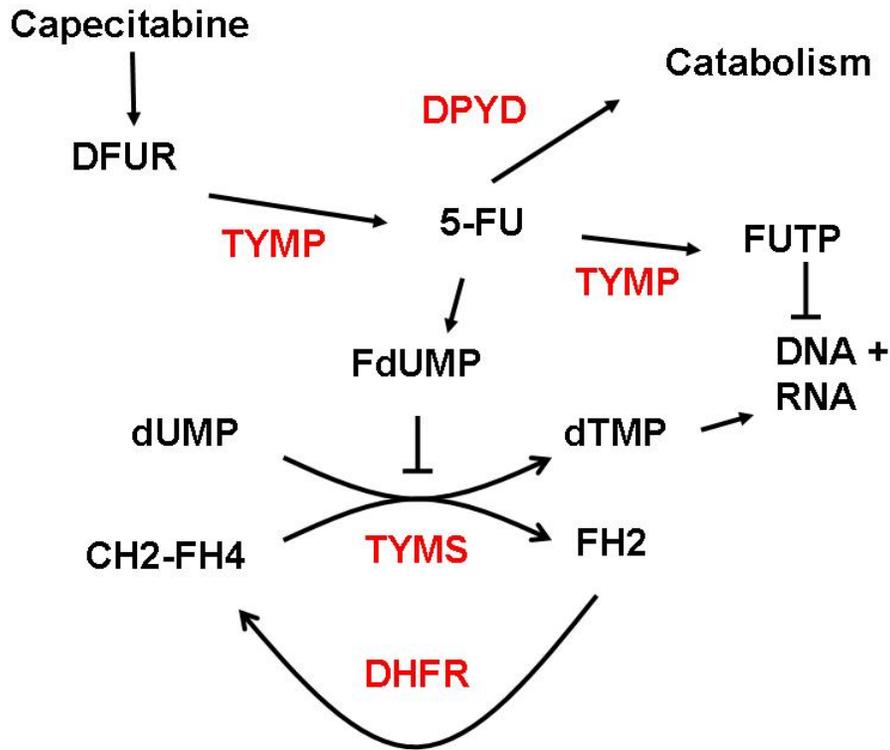
Figure 1: Pathway of capecitabine metabolism and catabolism. Abbreviations: CH2-FH4, 5-10 methylenetetrahydrofolate; DFUR, 5'-deoxy-5-fluorouridine; DHFR, dihydrofolate reductase; DPYD, dihydropyrimidine dehydrogenase; dTMP, deoxythymidine-5'-monophosphate; dUMP, deoxyuridine-5'-monophosphate; FdUMP, 5-flurodeoxyuridine-5'-monophosphate; FH2, dihydrofolate; 5-FU, 5-fluorouracil; FUTP, Fluorouridine triphosphate; TYMP, Thymidine phosphorylase; TYMS, Thymidylate synthase.

TYMS(PFS)

TYMS(TTP)
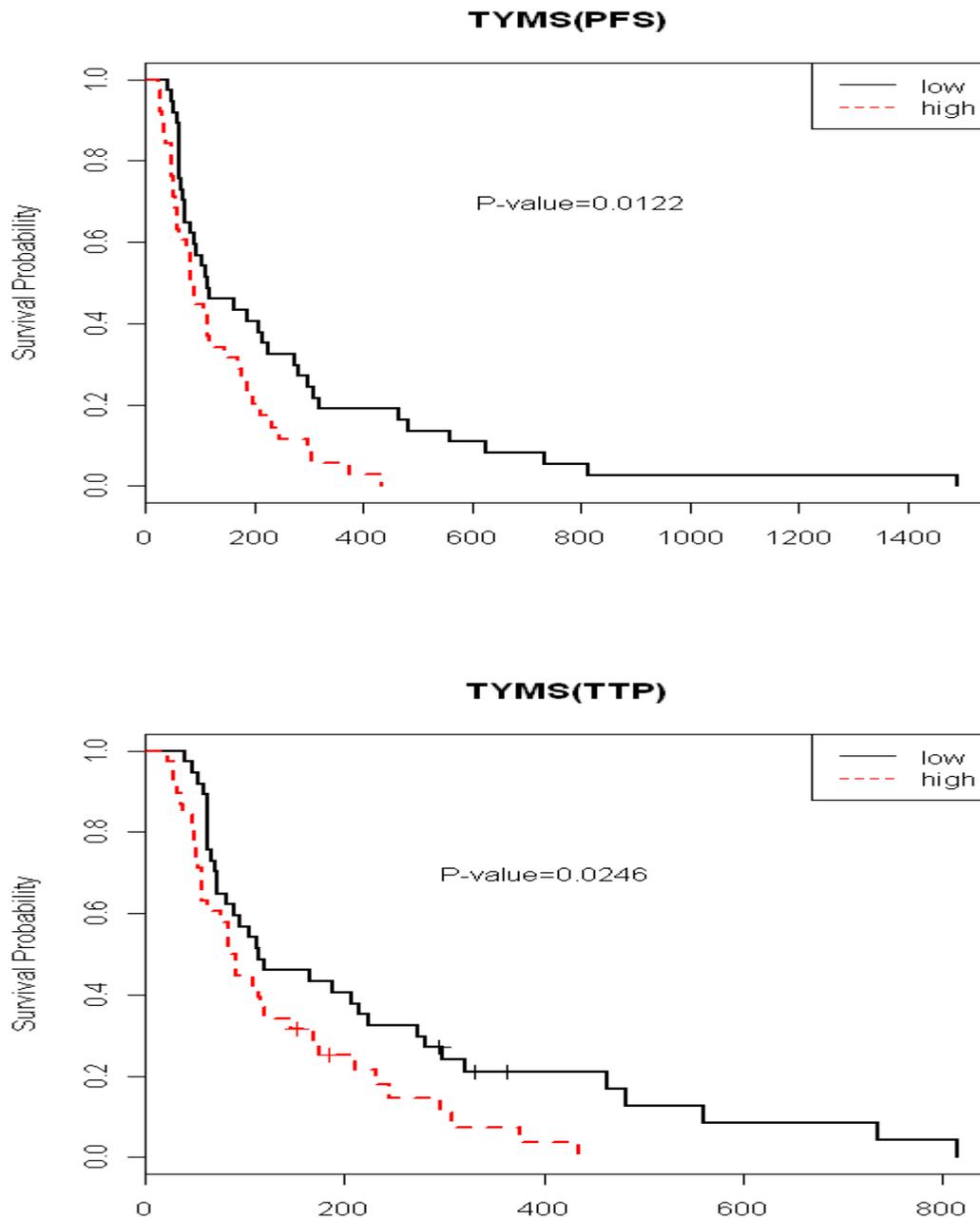
Figure 2:   Progression free survival (PFS) and time to progression (TTP) in the overall
patient population (n=75) dichotomized by the median into high (--) and low (-)
thymidylate synthase (*TYMS*) gene copy number using FISH probes.
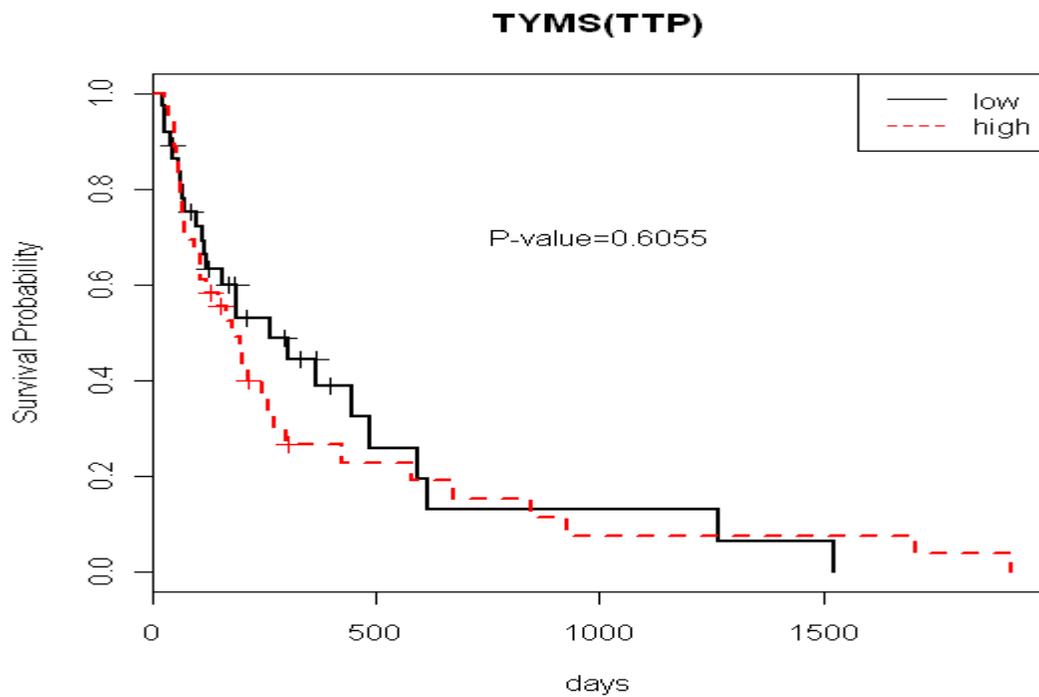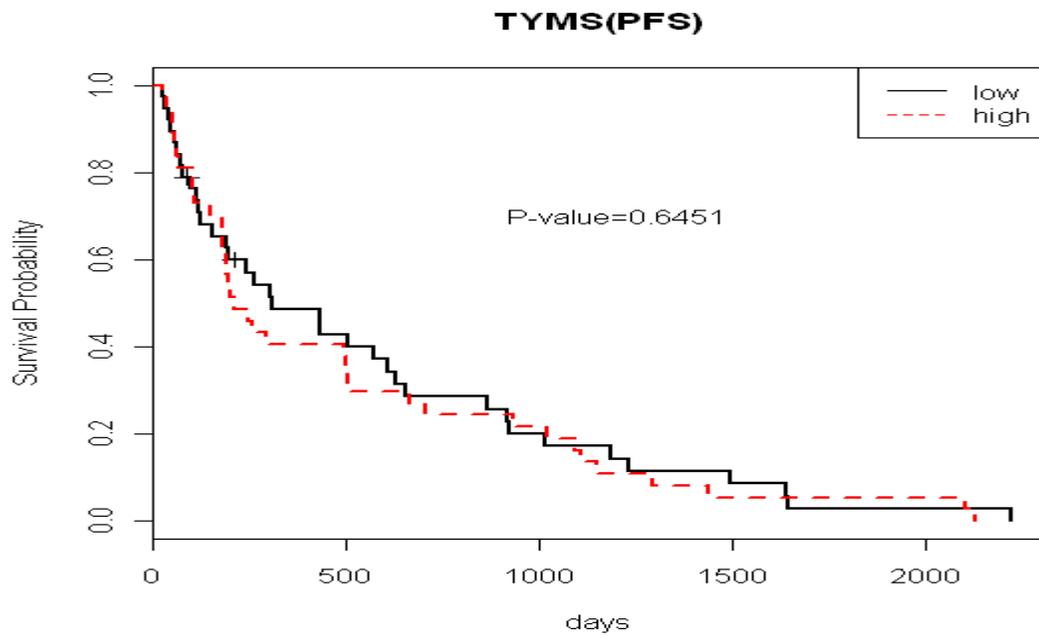
Figure 3: Progression free survival (PFS) and time to progression (TTP) in the overall patient population (n=73) dichotomized by the median into high (--) and low (-) thymidylate synthase (*TYMS*) gene expression using DASL.
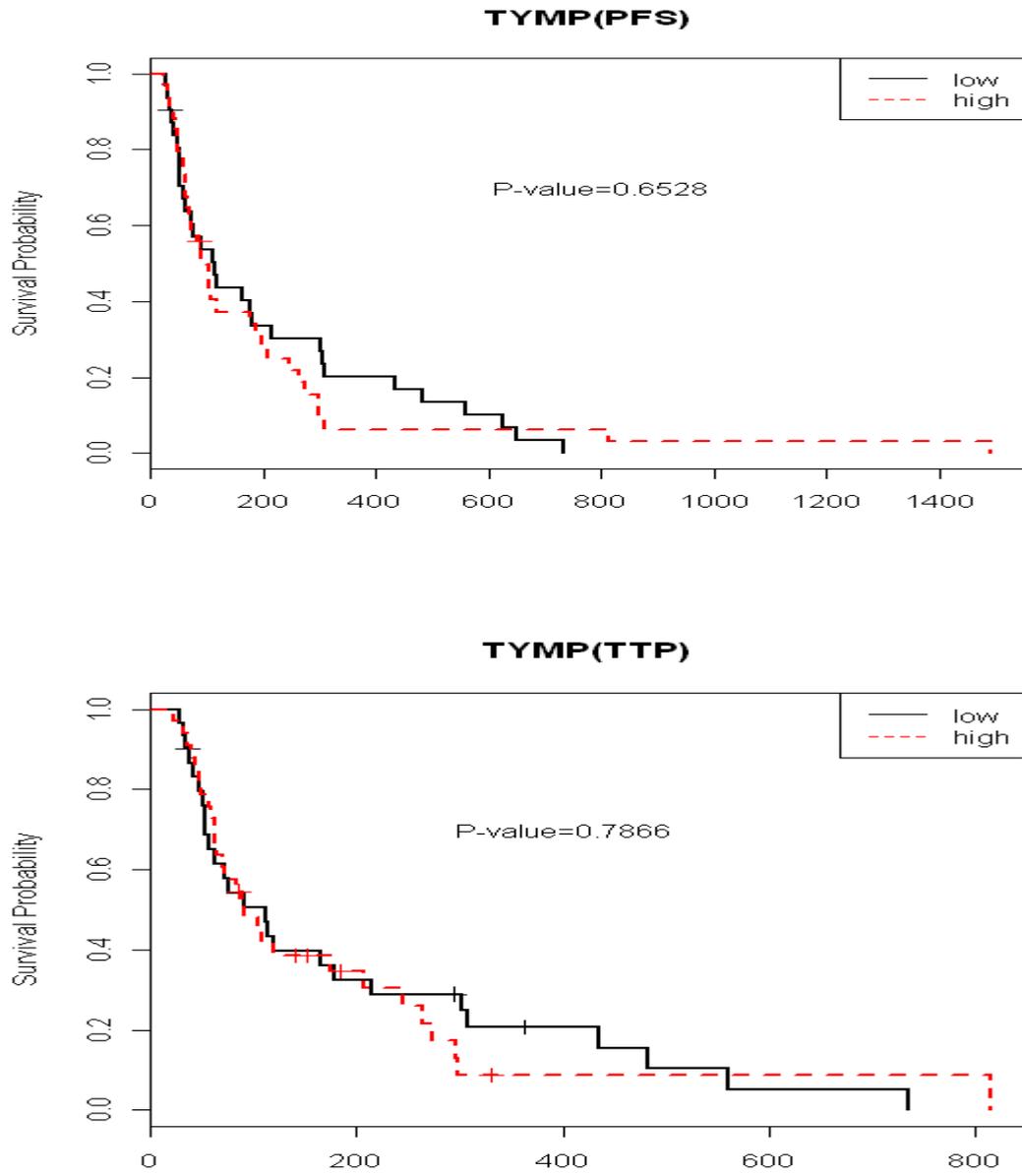
Figure 4: Progression free survival (PFS) and time to progression (TTP) in the overall patient population (n=75) dichotomized by the median into high (--) and low (-) thymidine phosphorylase (*TYMP*) gene copy number using FISH probes.
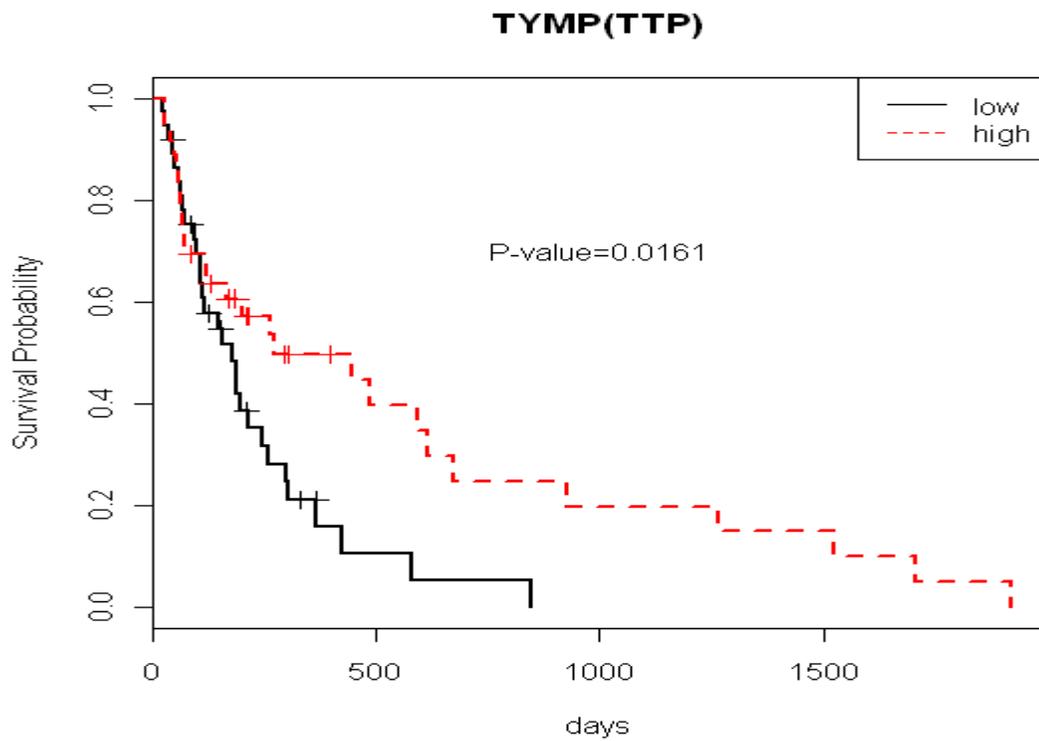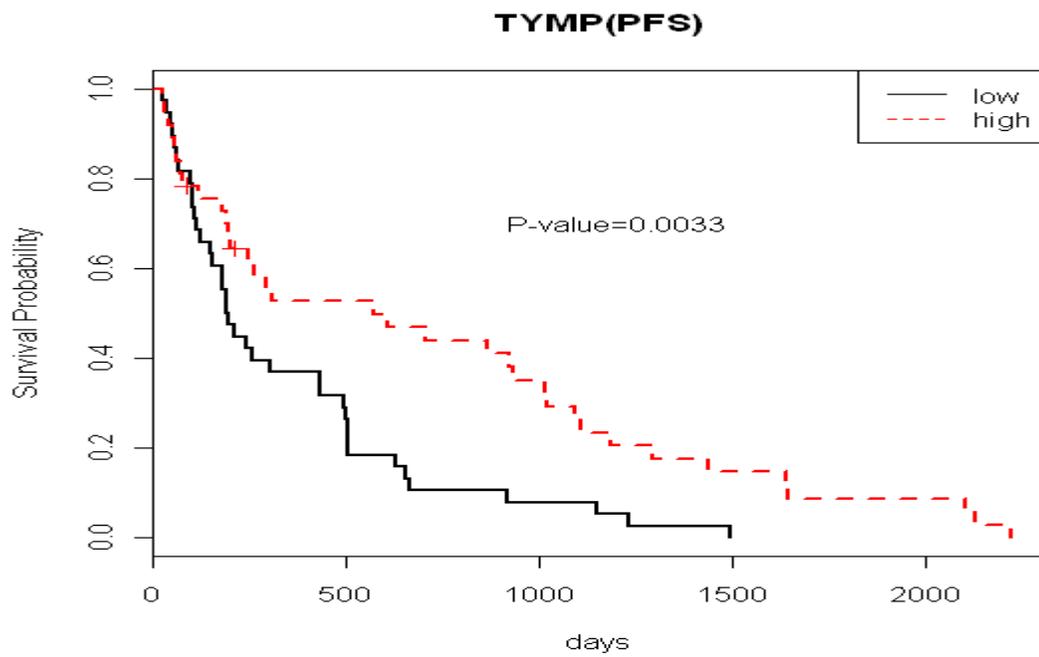
**Figure 5:** Progression free survival (PFS) and time to progression (TTP) in the overall patient population (n=73) dichotomized by the median into high (--) and low (-) thymidine phosphorylase (*TYMP*) gene expression using DASL.
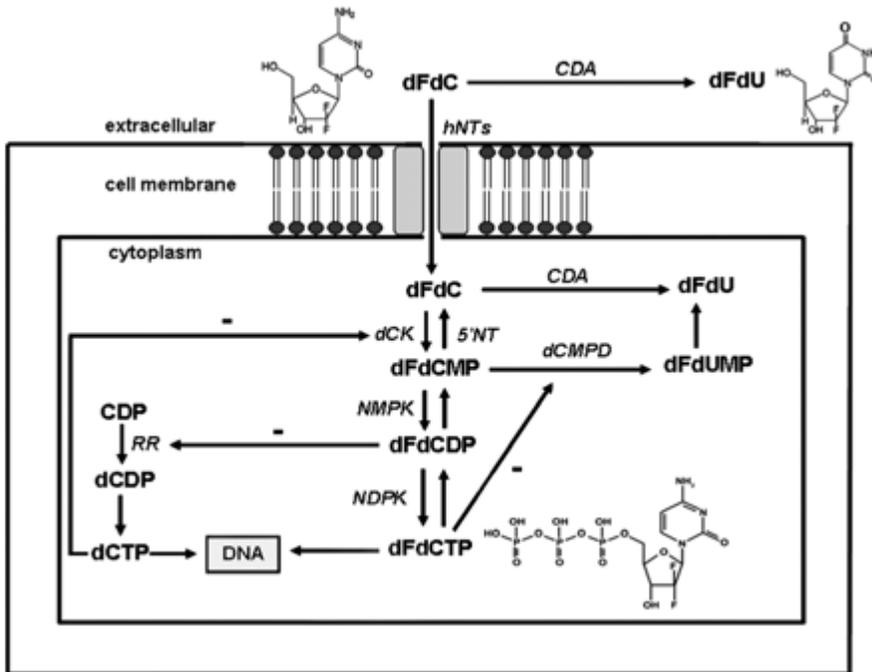
Figure 6:   Pathway of gemcitabine metabolism and catabolism. Abbreviations: 5'-NT, 5'-nucleotidase; CDA, cytidine deaminase; CDP, cytidine diphosphate; dCDP, deoxycytidine diphosphate; dCK, deoxycytidine kinase; dCMPD, deoxycytidylate deaminase; dCTP, deoxycytidine triphosphate; dFdC, 2',2'-difluorodeoxycytidine; dFdCDP, dFdC diphosphate; dFdCMP, dFdC monophosphate; dFdCTP, dFdC triphosphate; dFdU, 2',2'-difluorodeoxyuridine; dFdUMP, 2',2'-difluorodeoxyuridine monophosphate; hNTs, human nucleoside transporters; RR, ribonucleotide reductase.

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Patient Advocate Core: Conjoint Analysis Studies

Biomarkers offer the possibility of improving treatment decisions in oncology by permitting better predictions of treatment success based on individual cancer profiles, often using gene- or protein-based strategies. Indeed, several predictive biomarkers are widely used today, as exemplified by human epidermal growth factor receptor-2 (HER-2) overexpression and its indication for treatment of metastatic breast cancer with trastuzumab.[1]

Although few dispute the utility of biomarkers, they are seldom definitive indicators of events such as treatment response or cancer aggressiveness. Instead, biomarkers are associated with increased or decreased probabilities of cancer-related events (either efficacy or toxicity) that must then be weighed by patients and their healthcare teams in determining a course of action. As the development of predictive biomarkers proceeds, it is important to ask how patients weigh these probabilities and to examine the factors that influence treatment decision making.

Based on logic and introspection, we may expect biomarkers whose presence indicates a high likelihood of clinical response to significantly influence selection of that treatment. Conversely, we may expect biomarkers whose presence indicates severe and prolonged toxicity to a given therapy to meaningfully deter selection of that treatment. However, these are assumptions and they do not address specific questions such as: What level of predictive likelihood must the biomarkers show to be useful? What weight do patients ascribe to benefit vs. toxicity in treatment decision making? What demographic or disease-related characteristics are important in influencing treatment selection?

Methods: An online survey containing a conjoint analysis was e-mailed to members of breast cancer support organizations. The survey contained 14 different choice scenarios in which participants with a history of metastatic breast cancer were asked to choose between two treatment scenarios and whether or not they would undergo the treatment. The scenarios were designed based on

paclitaxel and capecitabine profiles related to medication format, likelihood of benefit, and side effects. The likelihood of benefit and likelihood/severity of side effects associated with these two drugs were varied based on the range of predictability afforded by ~~current~~ biomarkers.

Results

Demographics

A total of 641 participants responded to the survey: 307 from Metastatic Breast Cancer Network, 213 from Living Beyond Breast Cancer, 75 from Young Survival Coalition, and 46 from the BCMets listserv. Most of the respondents were Caucasian women who were married and had children, and had high levels of education and income (Table 1). A third of respondents had post-graduate degrees and 31% reported incomes of more than $100,000 per year (17% chose not to disclose income).

Of the 473 respondents with children, 59% had children over the age of 22, 17.8% had children 18-22 years of age, 22.6% had children 12-17 years, and 24.9% had children under age 12 (0.2% of respondents declined to specify their children's ages). Slightly less than half of the participants (47.1%) had to travel less than 30 minutes to their treatment site, 34.8% had to travel 30-60 minutes, 12.8% had to travel 1-2 hours, and 5.3% traveled >2 hours. On a quality of life

scale with ratings from 1=bad as it can be and 10=good as it can be, 48.3% of participants rated their quality of life as 8-10, 40.0% as 5-7, and 11.7% as 1-4.

Table 1. Participant Demographics and Time Since Diagnosis

Variable	% of Participants (N=641)
Women	99.7%
Caucasian	90.6%
Married	71.5%
Have children	73.8%
At least a 4-year college degree	70.2%
Age (years)	
Under 40	13.3%
40 to 49	25.9%
50 to 59	32.4%
60 to 69	24.0%
70 to 79	4.1%
80 or over	0.3%
Time since initial diagnosis	

<1 year ago	4.7%
1-4 years ago	38.5%
5-10 years ago	27.9%
>10 years ago	28.9%

Disease and treatment history

Most participants (72.4%) indicated that they were currently undergoing treatment and their disease was either stable or responding, 9.2% were in treatment but their cancer was not responding, and 15.3% indicated that their cancer was in remission. Participants had received treatment with capecitabine (43.5%), paclitaxel (47.6%), docetaxel (37.6%), and/or nabpaclitaxel (18.6%), or none of these (18.6%). Of the specific side effects about which participants were queried, fatigue (88.0%) was the most frequently experienced, followed by hair loss (71.0%), peripheral neuropathy (70.5%), cognitive problems (61.6%), diarrhea (60.5%), anxiety/depression (53.7%), nausea/vomiting (52.7%), and Hand-foot syndrome (47.3%).

Conjoint analysis

Results of the conjoint analysis indicate the predicted likelihood of choosing a treatment with the characteristics specified in each case. For each scenario, several variables are held constant (noted as "FIXED") to examine the pairwise tradeoffs (Figures 1-4).

<u>Benefit and toxicity</u>

The likelihood of participants stating that they would undergo a treatment was higher when the treatment was associated with a greater likelihood of benefit or lower toxicity (Figures 1 and 2). Respondents were more sensitive to benefit than to toxicity within the ranges tested, as demonstrated by the steeper declines in treatment choice as the likelihood of benefit decreased than as toxicity increased (Figures 1 and 2). Respondents showed a relatively high likelihood of taking treatment for any description of toxicity at the fixed levels of benefit tested (33% and 27%; Figures 3 and 4). Respondents also appeared much less likely to choose a treatment if the likelihood of benefit was 10% than if it was 30% or 50% (Figure 2). **When we say fixed level of benefit, we are talking about response rate, correct? We should say so.**

With regard to toxicity, the severity, duration, and type of side effect had modest effects on the choice to treat. With regard to peripheral neuropathy, increasing severity from moderate to severe or duration from during treatment to one year past treatment appeared to cause similar drops in likelihood of choosing treatment (Figure 3). The change from moderate to severe levels of diarrhea or Hand-foot syndrome appeared to have similar effects on likelihood of choosing treatment (Figures 3 and 4).

Figure 1. Percentage (+/- 95% errors) of respondents choosing to treat at varying levels of likelihood of treatment benefit and likelihood of experiencing moderate peripheral neuropathy for 1 year. The route of administration (intravenous) was held constant.

Fixed variable: IV administered			
Likelihood of Moderate PN for 1 yr			
	20%	40%	60%
20% Benefit LH	77.4	72.3	65.3
30% Benefit LH	89.8	86.4	81.6
50% Benefit LH	97.1	96.1	94.3
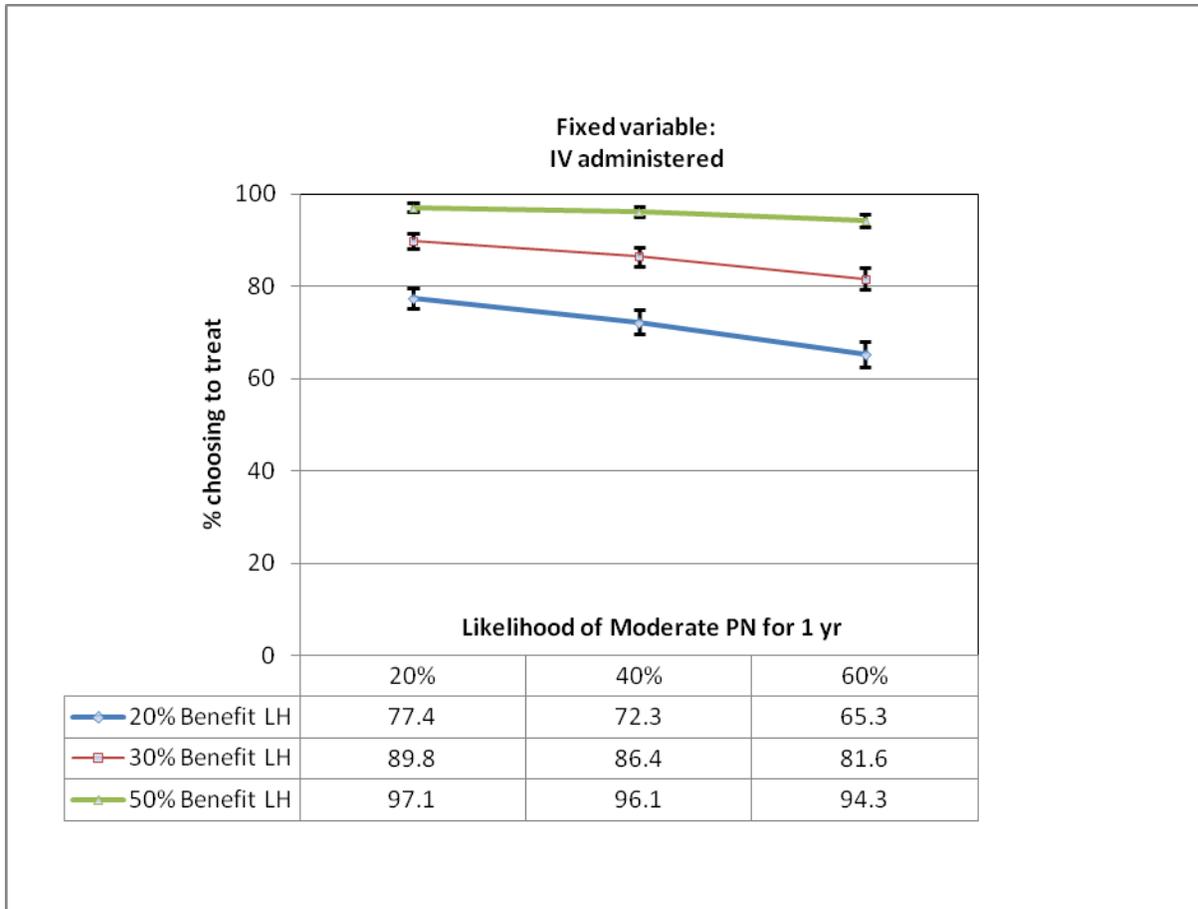
IV=intravenous, LH=likelihood, PN=peripheral neuropathy

Figure 2. Percentage (+/- 95% errors) of respondents choosing to treat at varying levels of likelihood of treatment benefit and likelihood of experiencing severe diarrhea during treatment. The route of administration (oral) was held constant.

	Likelihood of Severe Diarrhea During Treatment		
	10%	20%	40%
10% Benefit LH	51.9	49.3	43.8
30% Benefit LH	89.7	87.3	85.3
50% Benefit LH	98.1	96.1	94.6
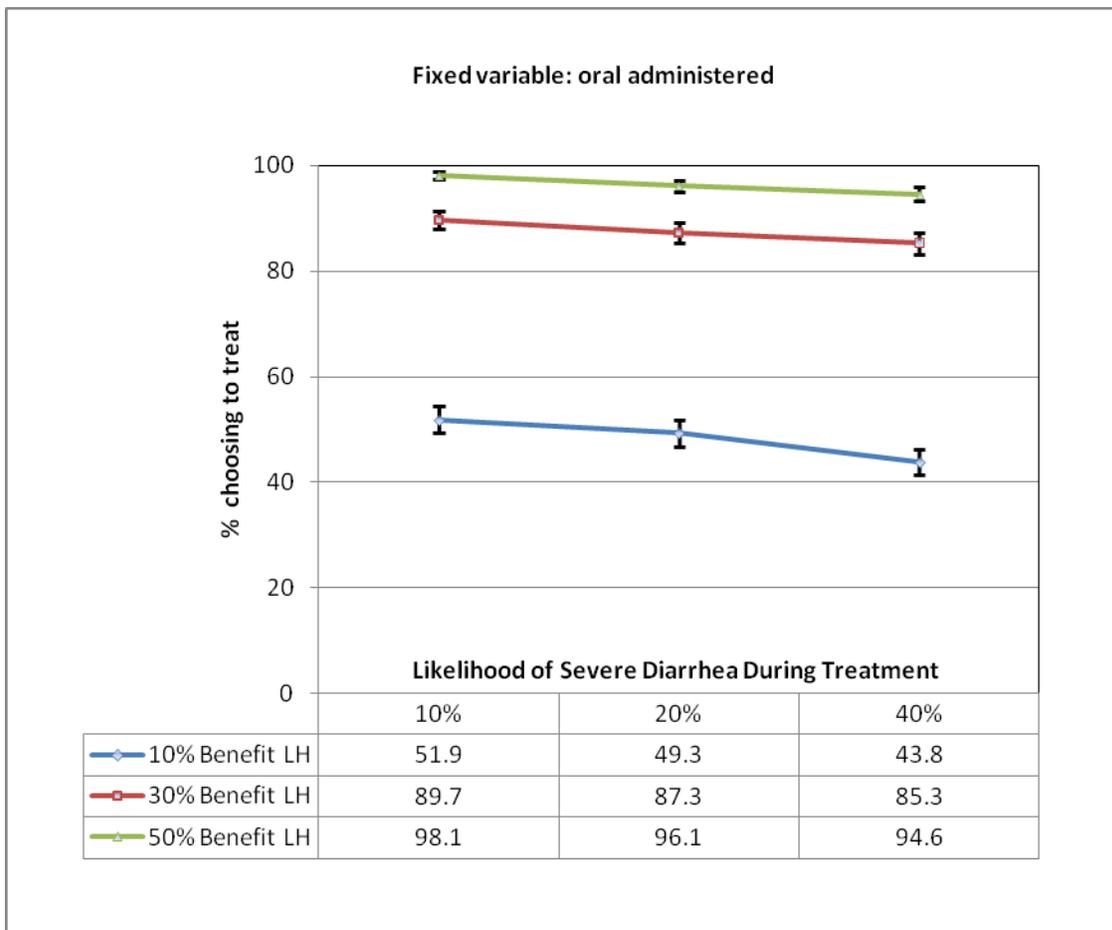
Figure 3. Percentage (+/- 95% errors) of respondents choosing to treat at varying levels of likelihood of toxicity (peripheral neuropathy) and severity/duration of toxicity. The route of administration (intravenous) and the likelihood of treatment benefit (33%) were held constant.

Fixed variables:
IV administered, 33% benefit likelihood

Severity/Duration of Peripheral Neuropathy

|	Moderate; during tx	Severe; during tx	Moderate; 1 year	Severe; 1 year	Moderate; rest of life
20% Tox LH	95.0	89.4	91.8	83.9	84.7
40% Tox LH	92.6	85.9	88.9	80.2	80.7
60% Tox LH	88.5	80.4	84.7	75.2	74.9
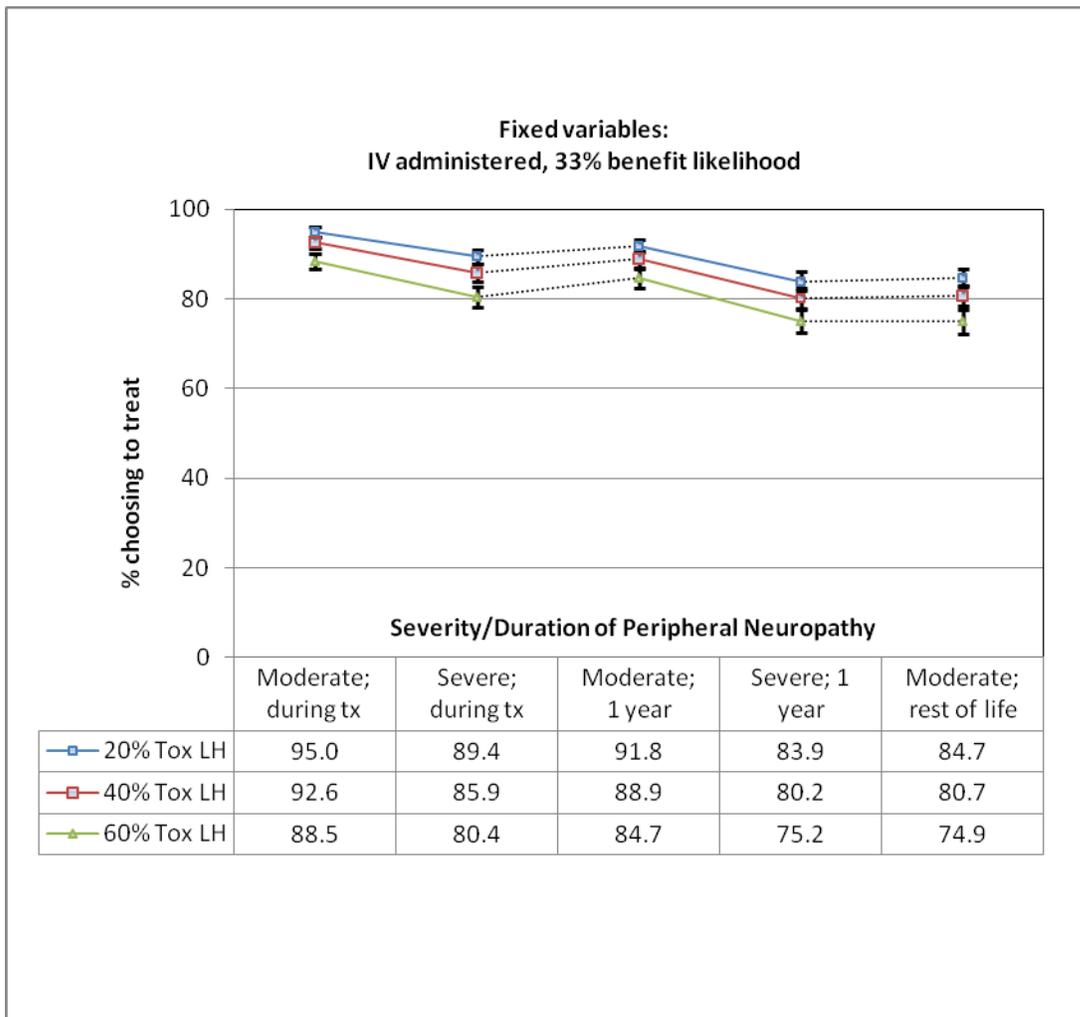
IV=intravenous, LH=likelihood, tx=treatment

Figure 4. Percentage (+/- 95% errors) of respondents choosing to treat at varying levels of likelihood of toxicity (diarrhea or Hand-foot syndrome) and severity/duration of toxicity. The route of administration (oral) and the likelihood of treatment benefit (27%) were held constant.

Don't like the figure, which joins 2 toxicities visually

Fixed variables: Oral administered, 27% benefit likelihood			
Severity/Type of Toxicity During Treatment			
	Moderate diarrhea	Severe diarrhea	Moderate HFS	Severe HFS
10% Tox LH	94.5	87.2	93.1	87.0
20% Tox LH	92.5	84.2	90.8	83.8
40% Tox LH	90.8	82.1	89.0	81.6
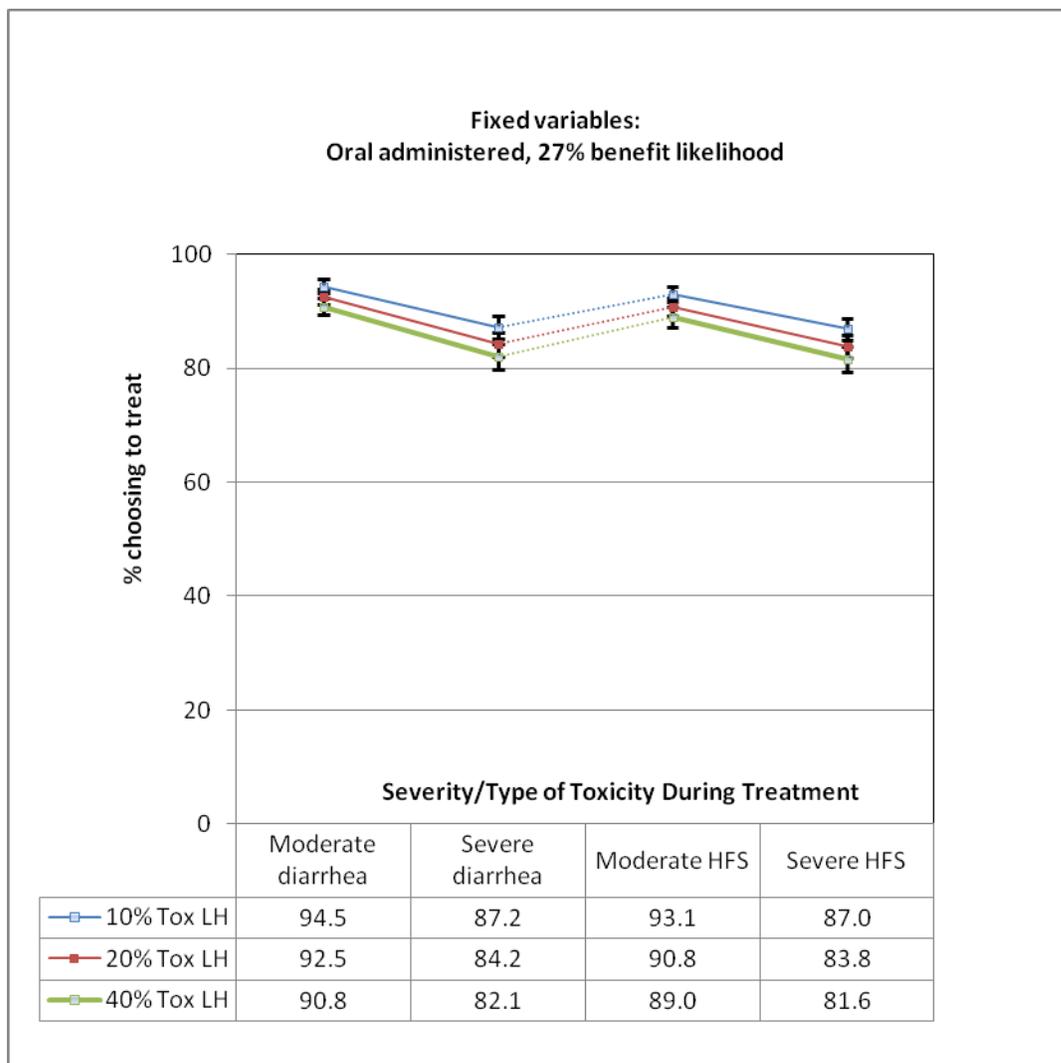
HFS=Hand-foot syndrome, LH=likelihood

Age

The respondent's age also had a significant effect on treatment choice. Respondents

under the age of 50 (n=251) showed higher likelihoods of choosing treatment than those

50 or older (n=390) for all levels of benefit (20%, 30%, and 50%) and all levels of toxicity

likelihood (20%, 40%, and 60%) (all P<0.05; Appendix). With the fixed variables of oral

administration and severe diarrhea, significant differences between the 50+ age group

and the younger groups were noted for 30% and 50% but not 20% likelihood of benefit.

With the fixed variables of oral administration and 27% likelihood of benefit, significant

differences between the 50+ age group and the younger groups were noted for severe

but not moderate toxicity. Although the effect of age was statistically significant in these

pairings, the percentages of older respondents choosing to treat in each scenario were

generally approximately 5-10% less than younger respondents.

Presence and age(s) of children

Respondents with children under the age of 18 generally had higher likelihoods of

choosing to take treatment, and those with children under age 12 have even higher

likelihoods in most iterations of the model. In contrast, no significant differences were

noted between respondents with adult children and those with no children (Appendix).

Again, the differences in percentages of respondents in all subgroups choosing to treat

in each scenario were generally within 5-10% of one another.   **Data**?

Proximity to treatment site

In some cases, respondents needing to travel 30 minutes or less to the treatment site showed significantly higher likelihoods of choosing to take treatment than those needing to travel longer than 30 minutes (Appendix). The differences in percentages of respondents in both subgroups choosing to treat in each scenario were generally within 5% of one another. **Data?**

Prior chemotherapy experience

Under some conditions, respondents who had previously taken capecitabine exhibited higher likelihoods of choosing to take a drug with a capecitabine profile, and those who had previously taken a taxane exhibited higher likelihoods of choosing to take a drug with a paclitaxel profile (Appendix). Statistically significant differences were observed much more consistently between the taxane vs. no taxane groups (23 of 25 pairings significant) than between the capecitabine vs. no capecitabine groups (9 of 21 pairings significant). The differences in percentages of respondents in both subgroups choosing to treat in each scenario were generally within 5-10% of one another. **Don't know what this paragraph means**

All subgroups

Across all subgroups, the only scenario in which respondents consistently showed approximately 50% or less likelihood of choosing treatment was when the fixed variables were oral administration and severe diarrhea during treatment, and the likelihood of treatment benefit was 20% (Appendix). In this scenario, the likelihood of respondents opting to take treatment ranged from 41.9% to 54.8% across all groups. However, when the fixed variables were oral administration and 27% likelihood of benefit, and the

likelihood of severe diarrhea during treatment ranged from 10% to 40%, many more respondents in each group chose to take the medication—the range was 79.4% to 92.0% across all subgroups.

Biomarker modeling

The results of the biomarker modeling are shown in Table 3. For both the paclitaxel and capecitabine toxicity biomarkers, most respondents are predicted to take treatment when the biomarkers are within the ranges tested and a smaller proportion of respondents (9.1% paclitaxel, 12.8% capecitabine) would not be expected to take treatment when the toxicity biomarkers are within the ranges tested. For the paclitaxel benefit biomarker, 75.7% of respondents would be predicted to take treatment when the biomarker is within the 20-50% range, and only 3.2% would not take treatment. Similarly, the majority of respondents are predicted to take capecitabine treatment when the benefit biomarker is within the range of 13-40%, and only 4.1% would not take treatment. **Confusing**

The third row of Table 3 shows the percentage of respondents who are predicted by the model to switch from no treatment to treatment or vice versa. These may be the individuals whose decisions may be influenced by biomarker information. These results indicate that benefit biomarkers in the ranges tested are predicted to have greater influence than toxicity biomarkers. Additionally, the capecitabine benefit biomarker is likely to show the highest degree of switching impact due to the range covered (13% to 40% benefit) as it spans important thresholds.

Table 3. Percentage of respondents predicted to choose or not to choose treatment at either end of the biomarker ranges specified in Table 2, or to choose treatment at the high end of the biomarker range and not to choose treatment at the low end of the biomarker range (ie, "switch)

	Paclitaxel toxicity biomarker	Paclitaxel benefit biomarker	Capecitabine toxicity biomarker	Capecitabine benefit biomarker
Take treatment	84.7	75.7	82.1	61.8
Do not take treatment	9.1	3.2	12.8	4.1
Switch	6.2	21.1	5.1	34.1

CONCLUSIONS: Likelihood of benefit was more important than toxicity for women considering treatment options for metastatic breast cancer.

% Choose to Treat
Benefit-Toxicity Combinations (N=414)
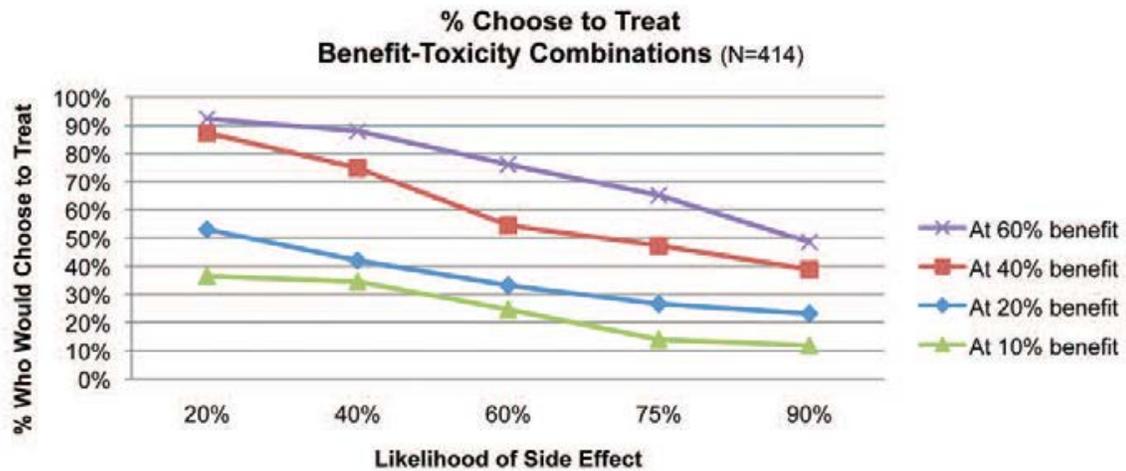
The above work was accepted for presentation at the 2011 Annual Meeting of the American Society of Clinical Oncology.

Reportable Outcomes

As mentioned above, the capecitabine predictive marker and the conjoint analysis evaluation of toxicity/benefit trade-offs were both selected for presentation at the 2011 and 2012 Annual Meetings of the American Society of Clinical Oncology.

Conclusion

During the current year we have completed tissue acquisition for the COE01 and COE05 projects, and completed accrual to the COE03 project. Initial analysis of FISH analysis for capecitabine suggests that thymidilate synthase may represent a valuable addition to predicting clinical benefit for this agent. In addition, this analysis suggests that the greatest predictive benefit occurs in estrogen receptor positive patients, suggesting a co-dependency between thymidilate synthase and estrogen receptor as predictive markers.

Or conjoint analysis work demonstrating patient preferences with regard to toxicity and benefit represents a novel approach to examining patient preferences in metastatic setting, leveraging both the internet and marketing techniques not generally utilized in oncology. Future analyses will explore patient preferences by examining specific toxicities associated with the drugs analyzed by the Center of Excellence.